
Appendix F

TRE Case Study: Central Contra Cost Sanitary District, Martinez, California, and Other San Francisco Bay Area POTWs

Abstract

TRE Goal:	No significant acute toxicity at 100% effluent
Test Organism:	<i>C. dubia</i>
TRE Elements:	TIE and source identification
Toxicants Identified:	Diazinon and chlorpyrifos
Toxicity Controls:	Multi-faceted public awareness program; ongoing program to identify and control sources; ongoing effort to identify POTW processes and operations that effectively remove organophosphate insecticides.

Summary

Acute toxicity to *C. dubia* was consistently detected in a POTW effluent. Application of Phase I, II, and III TIE procedures showed that the toxicity was caused by diazinon and one or more additional organophosphate insecticides. Follow-up studies, which required development of more sensitive analytical methods, showed that chlorpyrifos was present at levels that exceeded the NOEC in all effluent samples that were toxic to *C. dubia*. Influent and effluent monitoring studies of San Francisco Bay Area POTWs identified large differences in both influent loading and removal of the two insecticides between the POTWs. All the POTWs sampled achieved substantial removal of diazinon and chlorpyrifos from influent wastewater. Higher removal of both insecticides were generally associated with POTWs that had filtration treatment, extended mean cell residence times, chlorine contact times, and/or long retention in ponds. Source

identification studies showed that the majority of the influent mass loading of the two insecticides was from residential sources. A multi-faceted outreach program was initiated within the POTW service area. Monitoring of effluent toxicity and insecticide concentrations to assess the effectiveness of the public outreach program is on-going.

Key Elements

1. The organophosphate insecticides, diazinon, and more recently, chlorpyrifos, have been implicated as causes of toxicity to *C. dubia* in POTW effluents.
2. Published TIE procedures are available to identify organophosphate insecticide toxicity (USEPA 1991, 1992, 1993a, 1993b, 1996). Application of new methods and procedures assisted in providing a more quantitative assessment of the role of diazinon and chlorpyrifos in effluent toxicity.
3. Source identification studies at the CCCSD demonstrated that the majority of the diazinon and chlorpyrifos influent loading was from residential sources.
4. Regional influent and effluent monitoring studies demonstrated patterns in influent diazinon and chlorpyrifos loadings at the CCCSD, which suggest there were demographic differences in use and disposal practices for organophosphate insecticides.
5. A multi-faceted public outreach program was implemented in the POTW service area. The effectiveness of the program is being assessed by frequent measurements of influent and effluent levels of diazinon and chlorpyrifos and effluent toxicity tests.

6. Monitoring studies showed that San Francisco Bay Area POTWs achieve substantial removal of both diazinon and chlorpyrifos. The highest levels of removal are associated with systems that have filtration systems, extended MCRTs, and/or longer chlorine contact times.

Introduction

Permit Requirements

During 1990–1991, the CCCSD conducted an effluent toxicity characterization program in which 18 acute toxicity tests were performed. The effluent produced detectable acute toxicity to *C. dubia* in 12 of the 18 test events. The CCCSD's NPDES permit requires no significant acute toxicity at 100% effluent; therefore, a TRE study was required by the California State Water Quality Control Board, San Francisco Bay Region, to determine the causes and sources of the acute toxicity.

This study was performed in addition to the TRE study that addressed effluent toxicity caused by Cu (see Appendix B). The CCCSD was required to meet permit limits based on toxicity testing using both *C. dubia* and echinoderms.

Description of the Treatment Plant

A description of the treatment plant is presented in Appendix B.

Facility Performance Evaluation

As part of the TRE study, the CCCSD conducted an internal facility performance evaluation to determine if the treatment system was operating at design performance specifications. A review of all relevant operating parameters indicated that there were no obvious performance deficiencies. During this period, monthly effluent tests showed intermittent acute toxicity to *C. dubia*, but no toxicity was detected to juvenile *P. promelas* (15- to 60-day-old).

Toxicity Identification Evaluation

USEPA TIE methods were used as guidance in conducting the Phase I (1988a), Phase II (1988b) and Phase III TIE studies (1988c).

Phase I TIE – Toxicity Characterization

A total of five Phase I TIE studies were conducted with the CCCSD final effluent to characterize the class of the toxicant(s) responsible for the acute toxicity to *C. dubia*. Tests were 48–72 hours in duration and TIE treatments were not renewed during the tests. TIE

treatments were conducted on 100% effluent. The results, shown in Table F-1, indicated that the toxicity was consistently reduced by treatment with C18 SPE columns at pH_i (initial pH of the sample) and PBO addition. Treatments that produced a partial decrease in toxicity in two or more samples included adjustment to pH 3 and aeration. Treatments that consistently did not decrease toxicity included pH adjustments, sodium thiosulfate, EDTA, or graduated pH treatment.

The results of the Phase I TIE studies showed that acute toxicity was consistently reduced by the C18 SPE column treatment, which removes non-polar organic chemicals. The methanol eluates from the C18 SPE column were toxic when added to dilution water at a concentration equivalent to 1.5 times (1.5X) the concentration in the effluent sample. It is important to note that the 1.5X calculation assumes that the toxicity was completely removed from the effluent sample by the C18 SPE column and further, that the toxicity was completely recovered from the column in the methanol eluate.

PBO was effective in preventing acute toxicity to *C. dubia* in all five samples. PBO blocks the metabolic activation and subsequent toxicity of organophosphate insecticides, which require metabolic activation to exhibit toxicity (Ankley et al., 1991). The ineffectiveness of sodium thiosulfate and EDTA suggest that oxidants and/or cationic metals were not implicated in the toxicity. The results of the graduated pH test also suggested that ammonia did not contribute to toxicity. Overall, the Phase I TIE results indicated that the effluent toxicity was due to non-polar organic toxicant(s), specifically one or more organophosphate insecticides, which require metabolic activation to produce toxicity. Diazinon, a metabolically activated organophosphate insecticide, has been reported to cause toxicity in municipal effluents (Norberg-King et al., 1989; Amato et al., 1992); therefore, subsequent Phase II studies focused on identifying organophosphate insecticides. Effluent and diazinon-spiked laboratory water were used to determine if the TIE treatments produced similar effects.

Phase II – Toxicity Identification

A total of four effluent samples were processed in Phase II. PBO completely prevented toxicity in all four effluent samples, suggesting that metabolically activated organophosphate insecticides were responsible for the acute toxicity. The Phase I TIE showed that the toxicity could be both removed by and

Table F-1. Matrix of Results of Phase I TIE Conducted on Five Effluent Samples with *C. dubia*

Treatment	Reduces Toxicity Due To	Samples with Substantially Reduced Toxicity				
		1	2	3	4	5
C18 SPE column (pHi)*	Non-polar organics, metals	■	■	■	■	■
C18 eluate toxic	Confirms non-polar organics	■	■	■	■	■
PBO addition	Organophosphate insecticides	■	■	■	■	■
Filtration	Filterable toxicants	■		■		
Aeration	Volatile/oxidizable toxicants		■		■	
Adjustment to pH 3	Acid hydrolyzable toxicants	■	■		■	
Adjustment to pH 11	Base hydrolyzable toxicants					
Thiosulfate addition	Oxidants, some metals					
EDTA addition	Cationic metals					
Graduated pH test	Ammonia, metals					

* pHi = initial pH.

recovered from C18 SPE columns; therefore, the Phase II TIE procedures focused on the use of the columns to fractionate the sample for further characterization. Aliquots of the samples were concentrated on C18 SPE columns and the columns were eluted with a series of methanol:water mixtures (USEPA, 1993a). Acute toxicity tests were then conducted on each fraction at 1.5X the original effluent concentration.

The 75% fraction from all the effluent samples was acutely toxic. In some samples, adjacent fractions (e.g., 70, 80, and 85%) also exhibited acute toxicity. The toxic fractions were combined, concentrated, and sequentially fractionated using HPLC. For comparison, an analytical standard of diazinon was run immediately prior to each effluent sample HPLC run. A total of 30 fractions were collected during the HPLC linear gradient (30–100% methanol:water for 25 minutes with 5 minutes at 100% methanol). Each fraction was assayed at 1.5X the original effluent concentration with *C. dubia*, and toxic fractions were treated with PBO to ascertain the presence of organophosphate insecticides. This procedure was similar to that described by USEPA (1993a). The results are summarized in Table F-2.

The diazinon standard consistently produced acute toxicity in one fraction (19), and in one HPLC run,

toxicity also was observed in another fraction (18). All four effluent samples also produced acute toxicity in fraction 19 and occasionally in adjacent fractions (18 and 20).

As shown in Table F-2, in all cases, PBO provided protection against acute toxicity in the HPLC fractions in which toxicity occurred (18–20). However, PBO did not protect against the toxicity of fractions 12 and 13. The results of the PBO treatment of the toxic fractions suggested that one or more metabolically activated organophosphate insecticides, such as diazinon, had a role in the toxicity of all four effluent

Table F-2. Summary of TIE Phase II Results

Sample	Toxic Fractions
Diazinon (Runs 1–4)	18 ^{*†} , 19 ^{*†}
Effluent 1	18 ^{*†} , 19 ^{*†}
Effluent 2	12 [†] , 19 ^{*†}
Effluent 3	18 ^{*†} , 19 ^{*†} , 20 [‡]
Effluent 4	13 [†] , 19 ^{*†}

* PBO provided full protection against toxicity.

† PBO provided no substantial protection against toxicity.

‡ PBO provided partial protection against toxicity.

samples. Diazinon consistently eluted in the same fractions that were identified in the effluent samples; therefore, further studies focused on confirming the presence of diazinon in the HPLC fractions and refining procedures for the accurate determination of diazinon in effluent samples. This latter aspect was challenging because diazinon is toxic to *C. dubia* at low concentrations (LC50=0.26–0.58 µg/L) (USEPA, 1991; Ankley et al., 1991; Bailey et al., 1997), and the C18 SPE column extracts of the effluent samples contained numerous interferences which made analysis by gas chromatography (GC) problematic. Diazinon analysis generally followed procedures described by USEPA (1993a). Diazinon was quantitated by GC/MS using selected ion monitoring. The detection limit for this procedure in the CCCSD effluent matrix was 0.010 µg/L of diazinon.

Phase III – Toxicity Confirmation

The role of diazinon in the CCCSD's effluent toxicity was assessed using the correlation approach (USEPA, 1988c). The purpose of the correlation approach is to determine whether there is a consistent relationship between the concentration of the suspected toxicant and the degree of effluent toxicity. If the correlation is not robust, the role of the suspect toxicant in the effluent toxicity should be re-examined.

A total of seven CCCSD effluent samples collected during July and August 1992 were evaluated by comparing the expected toxicity based on diazinon (48-hour LC50=0.38 µg/L) with the measured effluent toxicity. The 48-hour toxicity of the effluent samples ranged from 1.25–2.17 TUa. Diazinon concentrations in these samples ranged from 0.120–0.280 µg/L, which corresponds to 0.32–0.74 TUa based on the 48-hour LC50 for diazinon (i.e., $0.12 \mu\text{g/L} \div 0.38 \mu\text{g/L}$ and $0.28 \mu\text{g/L} \div 0.38 \mu\text{g/L}$). The oxygen analog of diazinon (diazinon oxon) was not detected (<0.010 µg/L) in any of the effluent samples analyzed. Treatment of the toxic samples with PBO resulted in full reduction of toxicity in five samples, partial reduction in one sample, and no reduction in one sample. The effluent TUa and diazinon TUa values for the seven toxic samples are plotted in Figure F-1 along with the theoretical regression line, which depicts the case where all of the toxicity measured in the sample is due to diazinon (diazinon TUa = effluent TUa).

The linear regression of effluent TUa versus diazinon TUa had an R^2 value of 0.75 ($p \leq 0.01$), which indicates that diazinon concentrations can account for 75% of

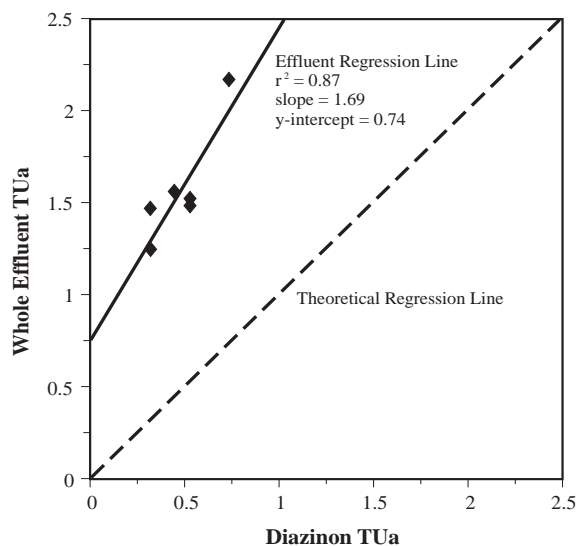


Figure F-1. Effluent TUs versus diazinon TUs in the CCCSD effluent samples.

the variability in the toxicity of the effluent samples. However, the regression is above the theoretical regression line, which suggests that either the analytical procedure for diazinon was consistently detecting less than the actual effluent concentration, and/or there were one or more additional toxicants present in the effluent samples. Further studies were undertaken to assess both possibilities.

Analytical procedures were reviewed by the CCCSD and were found to have acceptable levels of precision and accuracy. In an effort to identify the missing toxicant(s), more rigorous extraction procedures were applied to additional samples of effluent that were toxic to *C. dubia*. The effluent samples were exhaustively extracted with methylene chloride, evaporated to dryness, and resolubilized in hexane. Analysis of the extracts by GC/MS revealed the presence of chlorpyrifos, a metabolically activated organophosphate insecticide, in all the toxic effluent samples at concentrations greater than the NOEC of 0.030 µg/L (AQUA-Science, 1992; Bailey et al., 1997).

Follow-Up TIE Studies

Before further Phase III studies were initiated, a series of studies were conducted to validate the Phase I and II TIE findings for diazinon and to determine why the Phase II TIE process failed to identify chlorpyrifos as a toxicant in the CCCSD effluent. The results of these studies are summarized in Table F-3.

Table F-3. Summary of Follow-Up TIE Studies

TIE Treatment	Effect on Organophosphate Insecticides
pH adjustment	Diazinon is degraded rapidly at pH 3, but is relatively stable at pH 11
PBO addition	PBO at 100–700 µg/L effectively protects against three times LC50 concentration of diazinon and chlorpyrifos (1.6 and 0.24 µg/L, respectively). Effectiveness of PBO is affected by the matrix; therefore, use a range of PBO additions (USEPA, 1991a, 1993a).
C18 SPE	Diazinon is well recovered (80–100%) from C18 SPE columns Diazinon elutes sharply in specific methanol/water fractions: 75–80% methanol fractions for C18 SPE columns Chlorpyrifos is poorly recovered from C18 SPE columns (40–50% recovery) Chlorpyrifos tends to elute in broad bands: 80–95% methanol fractions for C18 SPE columns
HPLC fractionation	Diazinon is well recovered in specific fractions from C18 HPLC columns Recovery of chlorpyrifos from C18 HPLC columns is highly variable (20–60% recovery)
Sample stability studies	Significant amounts (20–40%) of diazinon and chlorpyrifos are lost from influent and effluent samples stored in either glass or plastic containers for 48 hours Effluent samples should be analyzed or extracted within hours of collection

The follow-up studies provided additional insight into the initial Phase I and II TIE results. The instability of diazinon at pH 3 is consistent with the reduction in effluent toxicity after pH 3 treatment. Diazinon is well recovered through the Phase II concentration and fractionation steps (Bailey et al., 1996); therefore, toxic fractions corresponding to those produced by diazinon standards should be present in all toxic effluent samples, as was demonstrated in the TIE.

On the other hand, the low overall recovery of chlorpyrifos from C18 SPE columns would explain the failure to detect chlorpyrifos toxicity in the effluent C18 SPE and HPLC fractions. For example, using the values in Table F-3, the recovery of chlorpyrifos in HPLC fractions could be as low as 8% (i.e., 40% recovery from 3 mL SPE column \times 40% recovery from 1 mL SPE column \times 50% recovery from HPLC column). This level of recovery would require an add-back of more than 12X to ensure that concentrations of chlorpyrifos in the HPLC fractions and the effluent samples were comparable. This study indicated that add-backs of fractions at levels substantially greater than 1.5X should be avoided because of the potential to amplify the toxicity due to toxicants that are below the toxic threshold in the effluent, but are well conserved through the TIE process. This could lead to erroneous identification of chemicals that do not have a causal role in the effluent toxicity.

A critical issue facing the investigator is how to identify toxicants that are not well recovered through the TIE process. Recently, procedures have been developed to selectively remove diazinon and chlorpyrifos from effluent samples using antibody-mediated processes (Miller et al., 1996; Miller et al., 1997). This process involves treating the effluent sample with the chemical-specific antibody preparation that selectively removes up to 95% of the target chemical (either diazinon or chlorpyrifos). By conducting effluent toxicity tests before and after the antibody treatment, the exact contribution of the target chemical to the overall toxicity can be determined. In addition, use of sequential antibody treatments to remove both diazinon and chlorpyrifos from the effluent matrix can indicate the extent to which toxicity is not due to either compound. The residual toxicity can be further characterized through the TIE.

Alternative Analytical Procedures

A major limitation of the TIE study was obtaining accurate and timely analytical information on levels of insecticides in effluent samples and TIE treatments. The GC/MS methods that were available involved tedious extractions, clean-up, and the use of expensive analytical equipment that was fully scheduled for compliance-related purposes. ELISA procedures were evaluated as an alternative analytical method for the analysis of diazinon and chlorpyrifos in subsequent Phase III TIE and source identification studies.

Commercially available ELISA kits (Beacon Analytical, Scaresborough, Connecticut) have some distinct advantages over GC or GC/MS methods, including cost (\$40–70 versus \$250–500 per sample), sample volumes (100 µL versus liters), sample turn-around (hours versus days or weeks), and equipment costs (\$3,000 versus >\$50,000). The detection limit for ELISA kits for diazinon and chlorpyrifos (0.030 µg/L) is also comparable to that for GC/MS. An interlaboratory study involving 6 laboratories and a total of 19 influent samples was conducted to compare the performance of ELISA, GC, and GC/MS procedures for diazinon and chlorpyrifos. The study showed that ELISA values for both insecticides were highly correlated ($R^2 > 0.95$) with GC and GC/MS results for those laboratories (Singhasemanon et al., 1997). The results were comparable over a wide range of concentrations (i.e., 0.030 to 31.5 µg/L for diazinon and 0.030 to 9.8 µg/L for chlorpyrifos).

Based on the excellent performance of the ELISA procedures in the interlaboratory study, ELISA procedures were used to monitor diazinon and chlorpyrifos concentrations in the CCCSD influent and effluent samples during follow-up studies, including source identification, POTW influent removal studies, and monitoring the effectiveness of public outreach programs.

Source Identification Studies

Source Study 1

A reconnaissance study was conducted in August 1995 to identify potential sources of diazinon and chlorpyrifos in wastewater from selected residential and commercial sources within the CCCSD collection system. A total of 36 24-hour composite samples of influent were analyzed for the two insecticides by ELISA. The samples included daily and/or hourly

composite samples collected from a residential community, and from selected businesses within the CCCSD collection system, including self-service pet grooming facilities, operations centers for pest control operators, and kennels.

The measured levels of diazinon and chlorpyrifos were coupled with estimated flows from the various sources to provide estimates of overall contribution of the two insecticides to the CCCSD's influent. The results are shown in Table F-4.

Diazinon and chlorpyrifos concentrations in the wastewater from the residential sources were highly variable (0.050–0.720 µg/L and <0.050–0.520 µg/L, respectively). Peak concentrations of both insecticides in the residential samples were measured in the samples collected on Saturday afternoon. The cause of the spikes of the insecticides in the residential wastewater is under further study and may be related to home use and/or improper disposal of these chemicals during weekend activities (e.g., lawn care operations for diazinon and pet flea control for chlorpyrifos).

Diazinon and chlorpyrifos levels in wastewater samples collected from commercial sources also were highly variable (<0.030–16.0 µg/L and 0.040–5.4 µg/L, respectively). The highest concentrations of both insecticides were measured in wastewater samples from a commercial kennel.

Overall, the reconnaissance study showed that although high levels of diazinon and chlorpyrifos were detected in some of the wastewater samples from commercial sources, the vast majority of the loading of the insecticides into CCCSD influent during the sampling period was from residential sources. This finding agrees with an earlier study of sources of diazinon in Fayetteville, NC (Fillmore et al., 1990).

Table F-4. Diazinon and Chlorpyrifos Concentrations in Wastewater Samples from Selected Residential and Commercial Sources in the CCCSD

Source	Diazinon		Chlorpyrifos	
	µg/L	% of Total Influent Loading	µg/L	% of Total Influent Loading
Residential	0.050–0.720	101	<0.05–0.52	94
Commercial: Pest control operators	<0.03–1.10	3	0.060–1.80	4
Pet groomers	<0.03–0.10	<1	0.04–7.00	2
Kennels	0.070–16.00	2	3.10–5.40	1

Source Study 2

Results of the reconnaissance study were used by the CCCSD and the California Department of Pesticide Registration (CADPR) to develop a plan for a more definitive study that was conducted from June to September 1996 (Singhasemanon et al., 1997). In this study, over 200 flow-proportional 24-hour composite samples were collected from each of 5 residential areas and 12 businesses (pet groomers, pest control operators, and kennels) within the CCCSD collection system. Flow measurements were made at selected sampling points in order to calculate mass loadings of diazinon and chlorpyrifos. The measured flows in residential areas were compared with modeled flow data obtained from a computer program [Sewer Network Analysis Program (SNAP) 1989, developed by the CCCSD]. The SNAP program applies modeled land use, groundwater infiltration, and CCCSD plant influent data to determine flow rates from the sampled areas. Concentrations of the insecticides were measured using ELISA, GC, and/or GC/MS procedures. The loading of diazinon and chlorpyrifos in the CCCSD influent from residential sources was estimated by multiplying the mean insecticide concentrations measured from the residential sites by the SNAP flow rates from the sampled sources. The commercial loading was estimated by multiplying the mean insecticide concentrations measured at each business by the measured flows and the number of similar businesses in the sewer service area. The data were analyzed using a computer program (SAS[®], SAS Institute, Inc, 1994, Version 6.1, Cary, North

Carolina), which calculated the Uniformly Minimum Variance Unbiased Estimator (UMVUE) for the mean influent loading concentrations for the insecticide (Singhasemanon et al., 1997). The mean UMVUE influent concentrations and associated loading for diazinon was 0.230 µg/L and 34.7 g/day, respectively. Corresponding values for chlorpyrifos were 0.145 µg/L and 15.0 g/day. The percentage of the total loading contributed by residential, commercial and unknown sources is shown in Figure F-2.

The CADPR study concluded that:

- Levels of diazinon and chlorpyrifos were highly variable in wastewater samples from both residential and commercial sources.
- Residential neighborhoods contributed the majority of diazinon and chlorpyrifos to the CCCSD's influent.
- Although relatively high concentrations of both insecticides were found at commercial sources, low flows from these sources resulted in relatively small mass loadings.
- A mass balance showed that a significant mass of chlorpyrifos and, particularly, diazinon was unaccounted for. Uninvestigated sources such as restaurants, nurseries, and industrial facilities should be sampled in future studies.
- Future source reduction strategies should focus on residential customers to identify and correct behaviors that contribute to disposal of organophosphate insecticides to the sewer system.

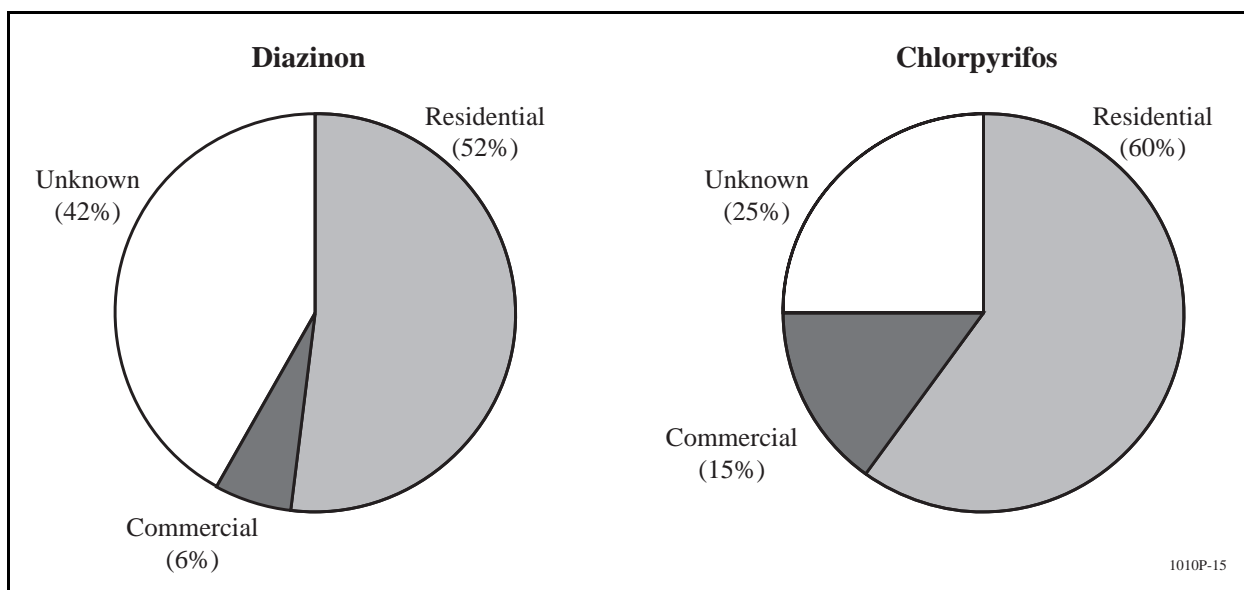


Figure F-2. Percent mass contribution of sources to the CCCSD influent.

As a result of the source identification studies, the CCCSD:

- Initiated a multi-faceted public outreach program targeting residential costumers to increase public awareness of the proper use of disposal of insecticides. The initial program included point of sale information sheets, newspaper articles, television ads, and billboards. A program to enhance public awareness of proper insecticide use by promoting integrated pest management practices is on-going.
- Shared study information with interested POTWs and State and Federal regulatory agencies.
- Initiated frequent effluent monitoring of diazinon and chlorpyrifos coupled with an effluent toxicity program to monitor the success of the public outreach program.
- Planned further studies to identify homeowner practices that contributed to the discharge of insecticides to the collection system.
- Reviewed disposal practices with pest control operators, pet care businesses, and kennels within the District.
- Conducted a study to identify the toxicity of alternative products for pet flea control.

Loading and Removal of Diazinon and Chlorpyrifos

Study 1

As an ancillary part of the CADPR source identification study, diazinon and chlorpyrifos were measured in seven consecutive daily samples of influent and effluent from CCCSD and two nearby POTWs [Union Sanitary District (USD), Fremont, California, and the Regional Water Quality Control Plant (RWQCP), Palo Alto, California]. The purpose of the study was to assess differences in loading and removal efficiencies for the POTWs. The three POTWs had similar influent flows (25–38 mgd), aeration detention times (3.8–5.6 hours), and clarifier detention times (2.0–4.2 hours). However, the CCCSD and the USD had shorter MCRTs (1.6–1.8 days versus 11.6 days) and shorter chlorine contact time (30–50 minutes versus 90 minutes) when compared to the RWQCP. In addition, the RWQCP treatment process incorporates two-stage aeration and dual media filtration to optimize particulate removal. The results of the study are shown in Figure F-3.

Daily concentrations of both diazinon and chlorpyrifos in the three POTWs varied widely during the sampling period. The CCCSD consistently had the highest

influent and effluent concentrations of both insecticides, followed by the USD and the RWQCP. The CCCSD and the USD, which have similar treatment processes, had similar removal efficiencies for diazinon (32 and 24%, respectively), and chlorpyrifos (53 and 49%, respectively). The RWQCP, which has longer chlorine contact time, two-stage aeration, and dual media filtration had the highest removal efficiencies for diazinon (82%) and chlorpyrifos (71%). The effect of these parameters on the removal and/or degradation of diazinon and chlorpyrifos in municipal influent was further evaluated in a subsequent study.

Study 2

A larger scale study was conducted to confirm the findings of the CADPR study, which suggested that there may be demographic and/or microclimatic differences in influent loadings of diazinon and chlorpyrifos to POTWs within the same region and moreover, there may be differences in removal efficiencies of the two insecticides in POTWs using different treatment systems. Seven daily 24-hour composite samples of influent and effluent were collected from 9 Bay Area POTWs during August 1997. The POTWs included the CCCSD and the cities of Fairfield-Suisun, Hayward, Palo Alto, Petaluma, San Francisco, San Jose, Union City, and Vallejo. Samples were analyzed for diazinon and chlorpyrifos within 24 hours of collection using ELISA (AQUA-Science, 1997). The results for diazinon and chlorpyrifos are shown in Figure F-4. Information on the characteristics of each POTW treatment system is shown in Attachment 1.

The results of this study confirmed and extended the findings of the previous study. A summary is provided below.

- Mean influent concentrations for both diazinon and chlorpyrifos were highly variable and ranged from 0.278–1.211 µg/L and 0.030–0.176 µg/L, respectively. These results suggest that there are regional demographic, and possibly, climatic differences in use and disposal practices for the insecticides.
- All the POTWs achieved substantial removal of the two insecticides from influent (up to 98% for diazinon and up to 86% for chlorpyrifos). These removal rates are generally higher for both insecticides than were observed in the previous study. The highest levels of removal were

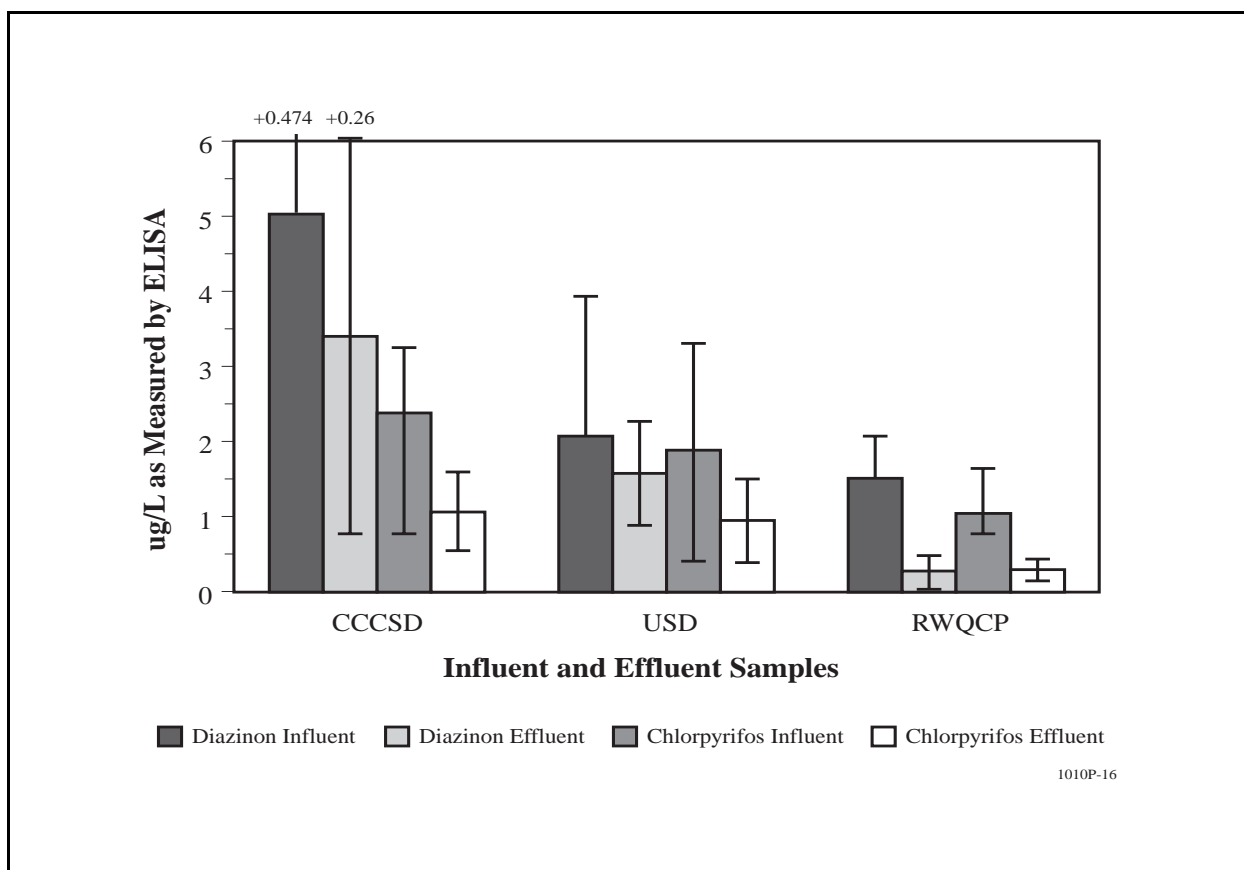


Figure F-3. Mean diazinon and chlorpyrifos concentrations (\pm std) in influent and effluent from three Bay Area POTWs.

associated with POTWs that had filtration, longer MCRTs and chlorine contact times, and long retention in ponds.

- Mean effluent concentrations for diazinon and chlorpyrifos ranged from <0.030 – 0.241 $\mu\text{g/L}$ and <0.030 – 0.085 $\mu\text{g/L}$, respectively. The combined mean effluent concentrations for both insecticides exceeded 1.0 TUa in only three of the nine POTWs sampled (including the CCCSD).
- Overall, the results showed that all the POTWs sampled during this period had potentially toxic levels of diazinon and chlorpyrifos in their influents. However, all the POTWs achieved substantial removal of both insecticides.

Another round of sampling was scheduled for February 1998 to assess seasonal effects on influent levels and removal rates from the POTWs.

Alternative Pet Flea Control Products

Toxicity source investigations by the CCCSD suggested that pet flea control products were a major source of chlorpyrifos in the influent (AQUA-Science 1995a and 1995b). Before the CCCSD could

recommend alternative products, it was necessary to conduct studies to determine the toxicity of several commonly used pet flea dips and shampoos. The acute toxicity of six flea shampoos and four dips was evaluated with *C. dubia* (AQUA-Science, 1995a; Miller et al., 1994). Although the products tested varied widely in toxicity, shampoos were generally less toxic than the dips. The most toxic products tested contained chlorpyrifos (IC₂₅s of 0.800 to 2.30 $\mu\text{g/L}$ as product), which were 2,500–7,000 times more toxic than the least toxic product tested, which contained D-limonene (IC₂₅ of 5.687 $\mu\text{g/L}$). The products containing pyrethrins and permethrin had intermediate levels of toxicity (IC₂₅s of 0.149–4.683 $\mu\text{g/L}$). Calculations (with the associated assumptions on use rate, system losses, and dilution) indicated that only flea dip products containing chlorpyrifos were sufficiently toxic to produce measurable effluent toxicity to *C. dubia*.

Effects of Household Bleach on Aqueous Concentrations of Diazinon and Chlorpyrifos

A study was conducted to determine if household bleach could be recommended to residential customers

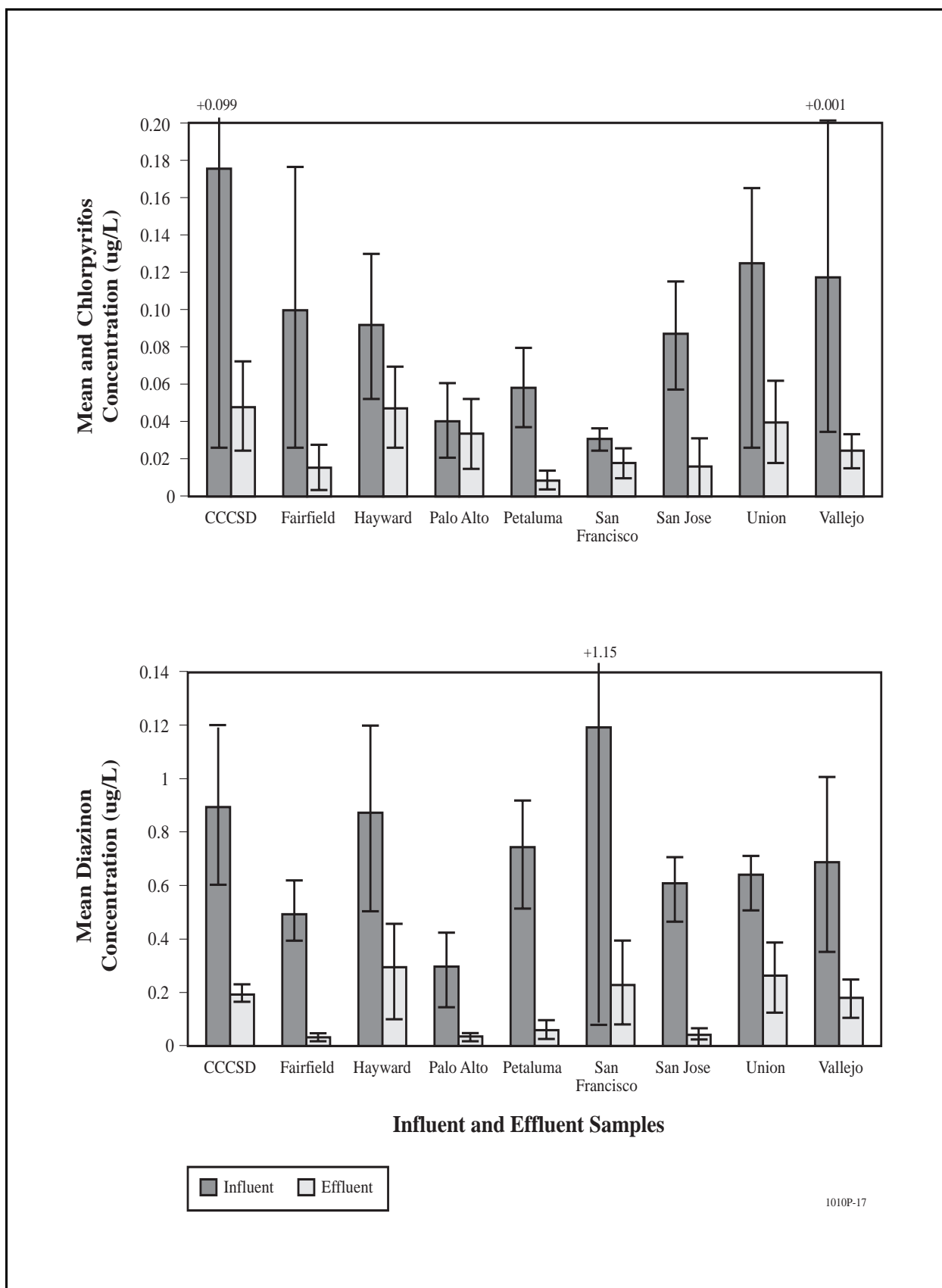


Figure F-4. Mean chlorpyrifos and diazinon concentrations (\pm std) in influent and effluent from nine Bay Area POTWs during August 1997.

as a measure to degrade diazinon in spray container rinsate and chlorpyrifos from pet flea washes prior to disposal into the sewer. Samples of tap water were spiked with high concentrations of diazinon (60.0 µg/L) and chlorpyrifos (10.0 µg/L) and treated with either 0.005 or 5% solutions of household bleach for 24 hours. After neutralization, concentrations of the insecticides were measured by ELISA (AQUA-Science, 1995a). Both bleach concentrations reduced concentrations of the insecticides by 86–92%. The study suggested that household bleach may be an effective pretreatment for waste solutions of diazinon and chlorpyrifos prior to disposal. Additional studies are planned to further define bleach exposure times and concentrations under actual use conditions, and to characterize the chemical oxidation products produced by the chlorine treatment.

Diazinon and Chlorpyrifos Concentrations in Water Samples from Restaurant Grease Traps

The CADPR source identification study recommended follow-up studies to determine concentrations of diazinon and chlorpyrifos in wastewater from restaurants. Water samples were collected from the grease traps of eight restaurants in the CCCSD service area (AQUA-Science, 1997). ELISA was used to measure concentrations of the two insecticides. Diazinon and chlorpyrifos concentrations ranged from 0.192–4.197 µg/L and 0.265–4.313 µg/L, respectively. The highest concentrations of both insecticides were found in wastewater from the same restaurant. The uses that contributed to these insecticide residues in the wastewater are currently being investigated by the CCCSD.

Regulatory Activities

Chlorpyrifos-Related

In January 1997, Dow-Elanco, as part of an agreement with USEPA, announced the following actions associated with the registered uses of chlorpyrifos (L. Goldman, USEPA Assistant Administrator for Prevention, Pesticides and Toxic Substances. Press Release on January 16, 1997):

- Withdrawal of chlorpyrifos from indoor broadcast and fogger flea control markets.
- Withdrawal of chlorpyrifos from direct application pet-care uses (shampoos, dips, and sprays).
- Increase marketing of ready-to-use products to replace concentrated formulas.
- Increase training and supervision of pest control operators.

- Revise chlorpyrifos labels to limit retreatment intervals.

If the chlorpyrifos in POTW influent loading is due to indoor and pet-care uses and/or misapplications by pest control operators, these actions should substantially reduce influent loadings of this chemical.

Diazinon-Related

In 1996, Novartis Crop Protection, Inc., the major U.S. registrant of diazinon, submitted voluntary label changes to USEPA to warn users not to dispose of this product into sanitary or storm drains. Novartis also developed educational materials with this message and provided the materials to selected cities in Texas and California. In 1997, Novartis completed a 4-year study with several POTWs in USEPA Region VI on diazinon occurrence and treatability (Novartis, 1997). A follow-up study is on-going with a California POTW to identify treatment processes that consistently optimize removal of diazinon (D. Tierney, personal communication, Novartis Crop Protection, 1997).

Discussion

In this case study, USEPA TIE procedures were used to identify organophosphate insecticide toxicity in a POTW effluent. Phase I and II TIE procedures identified diazinon as a candidate toxicant. Phase III TIE studies determined that effluent diazinon concentrations were significantly correlated with the extent of the effluent toxicity, but diazinon only accounted for approximately half of the effluent's toxicity. The follow-up TIE studies identified chlorpyrifos at potentially toxic concentrations in the toxic effluent samples. ELISA procedures were shown to provide sensitive and accurate measurements of the two insecticides in samples of POTW influent and effluent, and these procedures were used extensively in follow-up TIEs and source identification studies. Additional TIE experiments found chlorpyrifos to be poorly recovered through the Phase I and II TIE processes, which may explain why it has not been identified as a toxicant in other effluent TIEs.

The source identification studies at the CCCSD and other Bay Area POTWs showed that the influents contained highly variable, and often potentially toxic, levels of diazinon and chlorpyrifos, which appear to be originating primarily from residential rather than commercial sources. However, only a relatively small number of commercial sources have been sampled to date. Thus, it is possible that certain business types

(e.g., restaurants and nurseries) may be significant contributors of the two insecticides into wastewater. All of the POTWs that were sampled to date have demonstrated substantial removal of both insecticides from their influents. This was surprising because it was generally believed that these insecticides were poorly treated by POTWs (J.L. Miller, personal communication, Aqua-Science, Inc., Davis, California, April 1998). The available data suggest that there were substantial differences in influent loadings of diazinon and chlorpyrifos between POTWs within the San Francisco Bay region. Further studies are planned to explore the demographic basis for these differences to evaluate patterns of insecticide use. Seasonal trends in insecticide removal efficiencies are currently being monitored in nine Bay Area POTWs. Public outreach programs, supported, in part, by the manufacturers of diazinon and chlorpyrifos, have been implemented by the CCCSD and other POTWs across the country to increase awareness of the proper use and disposal of insecticides. Recent regulatory actions have resulted in the withdrawal of chlorpyrifos from the pet flea control market, and this action, coupled with the enhanced training of applicators and the increased use of prediluted insecticide products, may eventually reduce the influent loadings. Monitoring studies are in place at the CCCSD and elsewhere to determine if these programs will result in reduced influent loadings and decreased incidences of insecticide-related effluent toxicity.

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References

- Amato, J.R., D.I. Mount, E.J. Durham, M.T. Lukasewycz, G.T. Ankley, and E.D. Robert. 1992. An Example of the Identification of Diazinon as a Primary Toxicant in an Effluent. *Environ. Toxicol. Chem.* 11: 209-16.
- Ankley, G.T., J.R. Dierkes, D.A. Jensen, and G.S. Peterson. 1991. Piperonyl Butoxide as a Tool in Aquatic Toxicological Research with Organophosphate Insecticides. *Ecotoxicol. Environ. Saf.* 21: 266-74.
- AQUA-Science. 1992. *Phase III Effluent Toxicity Identification Evaluation Studies with Ceriodaphnia dubia*. Report for Central Contra Costa Sanitary District, Martinez, California.
- AQUA-Science. 1995a. *Diazinon and Chlorpyrifos in Wastewater from Residential and Commercial Sources*. Report for Central Contra Costa Sanitary District, Martinez, California.
- AQUA-Science. 1995b. *Flea Control Products Toxicity Assessment Study with Ceriodaphnia dubia*. Report for Central Contra Costa Sanitary District, Martinez, California.
- AQUA-Science. 1997. *Diazinon and Chlorpyrifos Concentrations in Wastewater Samples from Restaurant Grease Traps*. Report for Central Contra Costa Sanitary District, Martinez, California.
- Bailey, H.C., C. Digorgio, K. Krull, J. Miller, D. Hinton, and G. Starrett. 1996. Development of Procedures for Identifying Pesticide Toxicity in Ambient Waters: Carbofuran, Diazinon and Chlorpyrifos. *Environ. Contamin. Toxicol.* 15: 837-45.
- Bailey, H.C., J.L. Miller, M.J. Miller, L.C. Wiborg, L. Deanovic, and T. Shed. 1997. Joint Toxicity of Diazinon and Chlorpyrifos under Conditions of Acute Exposure to *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* 16: 2304-08.
- Fillmore, L.B., T.L. Morris, T.L. Champlin, M.C. Welch, and J.A. Botts. 1990. *Toxicity Reduction Evaluation at the City of Fayetteville Cross Creek Wastewater Treatment Plant*, Draft Report. Risk Reduction Engineering Laboratory, Cincinnati, Ohio. USEPA Contract No. 68-03-3431.
- Miller, J.L., H.C. Bailey, and M.J. Miller. 1994. Fate and Toxicity of Diazinon and Chlorpyrifos in Municipal Effluent. Published Abstracts. 15th Annual Meeting, Society of Environmental Toxicology and Chemistry, Denver, Colorado. October 30-November 3.
- Miller, J.L., M.J. Miller, and B. Silverman. 1996. Selective Removal of Diazinon and Chlorpyrifos from Aqueous Matrices. Published Abstracts. 17th Annual Meeting, Society of Toxicology and Chemistry, Washington, D.C., November 17-21.

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- Miller, J.L., M.J. Miller, B. Silverman, C. Foe, and V. DeVlaming. 1997. Selective Removal of Diazinon and Chlorpyrifos from Aqueous Matrices Using Antibody-Mediated Procedures. Published Abstracts. *18th Annual Meeting, Society of Toxicology and Chemistry*, San Francisco, California, November 16-20.
- Norberg-King, T.J., M. Lukasewycz, and J. Jenson, 1989. *Results of Diazinon Levels in POTW Effluents in the U.S.* NETAC Technical Report 14-89. USEPA, Duluth, Minnesota.
- Novartis Crop Protection and Makhteshim-Agan of North America. 1997. *Investigation of Diazinon Occurrence, Toxicity and Treatability of Southern United States Publicly Owned Treatment Works. Executive Summary.* Technical Report: 3-97. Greensboro, North Carolina.
- Singhasemanon, N., C. Nordmark, and T. Barry. 1997. *Diazinon and Chlorpyrifos in Central Contra Costa Sanitary District Sewer System, Summer 1996.* State of California Environmental Protection Agency, Department of Pesticide Regulation. Sacramento, California. Draft Report.
- USEPA. 1988a. *Methods For Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures.* EPA/600/3-88/034. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1988b. *Methods For Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures.* EPA/600/3-88/035. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1988c. *Methods For Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures.* EPA/600/3-88/036. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1991. *Methods for Aquatic Toxicity Identification Evaluations: Phase I, Toxicity Characterization Procedures.* Second Edition. EPA/600/6-91-003. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1992. *Toxicity Identification Evaluations: Characterization of Chronically Toxic Effluents, Phase I.* EPA/600/6-91-005F. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1993a. *Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity.* EPA/600/R-92-080. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1993b. *Methods for Aquatic Toxicity Identification Evaluations. Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity.* EPA/600/R-92-081. National Effluent Toxicity Assessment Center, Duluth, Minnesota.
- USEPA. 1996. *Marine Toxicity Identification Evaluation (TIE) Guidance Document, Phase I.* EPA/600/R-96/054. Office of Research and Development, Washington, D.C.

Attachment I
Summary of POTW Treatment System Characteristics

CCCSO

39 mgd
Primary sedimentation
Air-activated sludge (MART 1.6 days)
Secondary clarification
UV disinfection

Fairfield

13 mgd
Primary sedimentation
Oxidation towers with clarification
Air-activated sludge (MART 12–14 days)
Secondary clarification
Tertiary filtration with dual media
Chlorine disinfection (90–120 minutes)

Hayward

12 mgd
Valuators
Primary sedimentation
Fixed film reactors (sludge age n/a)
Anaerobic digester
Final clarifiers
Chlorine disinfection (~100 minutes)

Palo Alto

26 mgd
Primary sedimentation
Fixed film reactor to mixed aeration basins with
activated sludge (MART 11.6 days)
Secondary clarifiers
Mixed media filtration
Chlorine disinfection (90 minutes)

Petaluma

6 mgd
Primary clarification
41% to activated sludge

32% to trickling filter
27% bypasses to ponds where retention time is about
100 days

San Francisco

17 mgd
Primary sedimentation
Air-activated sludge (MART ~ 0.86 days)
Secondary clarification
Sodium hypochlorite disinfection

San Jose

137 mgd
Primary sedimentation
Air-activated sludge (MART ~ 4 days)
Secondary clarification
Nitrification and clarification (MART ~ 11 days)
Tertiary filtration with backwash to clarification (for
flow equilibrium)
Chlorine disinfection (40–60 minutes)

Union

31 mgd
Primary sedimentation
Air-activated sludge (MART ~ 1.75 days)
Secondary clarifiers
Chlorine disinfection (30 minutes)

Vallejo

12 mgd
Primary sedimentation
Biological filters
Aeration basins (MART ~ 3 days)
Clarification
UV disinfection and sodium hypochlorite contact
(8 minutes)